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PII: S0308-8146(17)31006-3
DOI: http://dx.doi.org/10.1016/j.foodchem.2017.06.024
Reference: FOCH 21246

To appear in: *Food Chemistry*

Received Date: 4 April 2017
Revised Date: 2 June 2017
Accepted Date: 5 June 2017

Please cite this article as: Chu, W., Gao, H., Chen, H., Fang, X., Zheng, Y., Effects of cuticular wax on the postharvest quality of blueberry fruit, *Food Chemistry* (2017), doi: http://dx.doi.org/10.1016/j.foodchem.2017.06.024

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Effects of cuticular wax on the postharvest quality of blueberry fruit

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ABSTRACT

The blueberry fruit has a light-blue appearance because its blue-black skin is covered with a waxy bloom. This layer is easily damaged or removed during fruit harvesting and postharvest handling. We investigated the effects of wax removal on the postharvest quality of blueberry fruit and their possible mechanisms. The removal of natural wax on the fruit was found to accelerate the postharvest water loss and decay, reduce the sensory and nutritional qualities, and shorten the shelf-life. Wax removal decreased the activities of antioxidant enzymes and contents of antioxidants, and accelerated accumulation of ROS and lipid peroxidation, especially at the later period of storage. Moreover, the organellar membrane structure was disrupted in fruit with wax removed. These results indicate that cuticular wax plays an important role in maintaining the postharvest quality and delaying fruit senescence. The results should improve our understanding for better preservation of postharvest quality of blueberry fruit.

Keywords: Blueberry fruit, Wax removal, Postharvest quality, Reactive oxygen species, Membrane structure

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1. Introduction

The outermost layer of plants is covered with cuticular wax, which is visible as a white or bluish coating on the surface of some fruits, such as blueberry, plum, and grape (Saftner, Polashock, Ehlenfeldt, & Vinyard, 2008; Wisuthiphaet, Damerow, & Blanke, 2014). Cuticular wax is the first protective barrier against biotic and abiotic stresses; it plays a vital role in limiting the non-stomatal water loss and in preventing the spore germination of pathogenic microbes (Bernard & Joubès, 2013; Samuels, Kunst, & Jetter, 2008). In recent years, several studies have demonstrated that cuticular wax is closely related to the postharvest quality of fruit (Lara, Belge, & Goulao, 2014; Martin & Rose, 2014). A sharp increase in postharvest water loss was observed upon the removal of wax from the surface of fruits, such as citrus and European plum (Mukhtar, Damerow, & Blanke, 2014; Wang et al., 2014). In addition, cuticular wax could inhibit spore germination and mycelial growth of *Alternaria alternata* in Asian pear fruit (Yin et al., 2011).

Blueberry (*Vaccinium* spp.) is widely popular among consumers because of its high nutritive value and pleasing flavor. The light-blue appearance of blueberry is because of its blue-black skin, which is covered with a waxy bloom. However, this conspicuous wax layer is vulnerable to damage, and it might be removed during the harvesting, packaging, and transport of fruit, resulting in unattractive appearance and giving the impression of poor fruit quality (Mukhtar et al., 2014). It is unclear whether the removal of wax affects the blueberry quality during storage.

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In general, various abiotic stresses, such as wound, cold, drought, and salt can disturb the cellular homeostasis and enhance the production of reactive oxygen species (ROS) in plants (Gill & Tuteja, 2010; Mittler, 2002). ROS are highly reactive and toxic, and can cause damage to lipids, proteins, carbohydrates, and DNA, eventually leading to oxidative stress (Gill et al., 2010; Mittler, 2002). The mitochondria, being the primary sites of endogenous ROS generation, are susceptible to oxidative damage (Sweetlove et al., 2002). The accumulation of ROS in cells could cause oxidative damage of the mitochondrial proteins, finally resulting in the mitochondrial dysfunction or cell death (Tian, Qin, & Li, 2013). It has been demonstrated that ROS initiate fruit senescence, and thereby, directly affect the postharvest quality of fruit (Qin, Wang, Liu, Li, & Tian, 2009; Tian et al., 2013; Xia, Chen, Qin, Li, & Tian, 2016). To some extent, the damage or removal of blueberry wax by mechanical abrasion during harvesting and postharvest handling is ineluctable. However, until date, there are no studies on whether the removal of wax layer is related to ROS metabolism and fruit senescence, and affects the postharvest quality.

In this context, the present study was conducted to assess the roles of cuticular wax on the postharvest quality of blueberry fruit during cold storage, by removing the wax. We also investigated the possible mechanism for the observed roles of cuticular wax with respect to the ROS metabolism.

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2. Materials and methods

2.1. Blueberry samples and treatment

Mature blueberry fruit (*Vaccinium ashei* 'Britewell') were harvested from a local blueberry orchard in Zhejiang Province of China on July 23, 2015. In this study, mature fruit were considered as those that remained for a maximum of 3 days on the vine after attaining the maximum blue color. The fruit were carefully handpicked with polyvinyl chloride gloves and were directly placed in plastic baskets. They were then transported within 2 h to the laboratory at about 10 °C in a refrigerated car. The fruit with no physical damage and uniform size and color were selected for the subsequent experiments.

To mimic the damage to the wax layer on the fruit surface during commercial handling, this layer was removed by slightly wiping with Blu-tack adhesive, instead of immersing the fruit in an organic solvent. The visual appearance and scanning electron microscope (SEM) images taken before and after the wax removal are shown in Fig. 1A. The natural wax layer of blueberry fruit contained a large amount of tubular wax crystals; after the removal of epicuticular wax, the surface became smoother. Both the untreated fruit (control group) and those with the wax removed were sub-packaged into 60 plastic clamshells (125 g), containing 50 fruit each, and then wrapped in 0.05-mm thick unsealed polyethylene bags. All the fruit were stored at 4 °C in about 90 % relative humidity for 36 d. Each treatment had three replicates.

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2.2. Measurement of the quality parameters

The color of the blueberry surface was determined by a chroma meter CR-400 (Konica Minolta Sensing Inc., Japan). The CIELAB software was employed to measure the L^*, a^*, and b^* values. The L^* value represents the lightness from black ($L^* = 0$) to white ($L^* = 100$), the a^* value indicates the color from green (-) to red (+), and the b^* value indicates the color from blue (-) to yellow (+). Twenty blueberry fruit were randomly selected from each treatment to measure the surface color.

The firmness of blueberry fruit was determined using a TA.XT Plus Texture Analyzer (Stable Micro Systems Ltd., U.K.) with a 5 mm diameter stainless steel probe. Twenty blueberry fruit were randomly selected from each treatment to measure the firmness. Each fruit was equatorially compressed by 5 mm at a test speed of 1.0 mm s^{-1} and the maximum force (N) during the test was recorded.

For the assessment of total soluble solids (TSS) and titratable acidity (TA), three replicate samples (8 fruit each) were wrapped in gauze and squeezed until no more juice was released. TSS was determined with a digital hand-held refractometer (Atago PAL-1, Japan). TA was determined by titration with 0.05 M NaOH to pH 8.2, and the result was expressed as a percentage of citric acid.

The weight loss was determined by weighing the blueberry fruit before and after the storage period and was expressed as the percentage of weight loss compared to the initial weight.

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The decay incidence was evaluated by the number of decayed fruit relative to the total number of fruit. The decayed fruit were considered as rotten and had visible fungal growth or bacterial lesions on their surface.

2.3. Measurement of ascorbic acid and total phenolic and anthocyanin content

The ascorbic acid content was determined using a colorimetric method with 4, 7-diphenyl- l, 10-phenanthroline according to the method described by Arakawa, Tsutsumi, Sanceda, Kurata, and Inagaki (1981), by measuring the absorbance at 534 nm based on a standard curve. The results were expressed as g kg^{-1} of ascorbic acid on a fresh weight basis.

The total phenolic content was detected with Folin–Ciocalteu reagent according to the method described by Zheng, Wang, Wang, and Zheng (2003). The results were expressed as g kg^{-1} of gallic acid equivalents on a fresh weight basis.

The total anthocyanin content was measured using the pH differential method as described by Correa-Betanzo, Allen-Vercoe, McDonald, Schroeter, Corredig, and Paliyath (2014). The results were expressed as g kg^{-1} of cyanidin-3-glucoside equivalents on a fresh weight basis.

2.4. Determination of $O_2^{\cdot-}$ and H_2O_2

The rate of production of superoxide anion ($O_2^{\cdot-}$) was determined according to the method described by Li, Long, Gao, Han, Jin, and Zheng (2017). The results were calculated using $NaNO_2$ as a standard and were expressed as μmol kg^{-1} s^{-1} based on fresh weight.

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The H_2O_2 content was determined with Hydrogen Peroxide assay kit (Nanjing Jiancheng Bioengineering Institute, China) by measuring the absorbance at 405 nm. The results were calculated using H_2O_2 as a standard and were expressed as mmol kg^{-1} based on fresh weight.

2.5. Assays of the activities of antioxidant enzymes

The extraction of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) was performed according to the procedure of Chen, Gao, Fang, Ye, Zhou, and Yang (2015), and ascorbate peroxidase (APX) extraction was carried out according to Han, Li, Jin, Li, Wang, and Zheng (2017).

The SOD activity was assayed as described previously (Chen et al., 2015). The results were expressed as U kg^{-1} fresh weight, where one unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of nitroblue tetrazolium (NBT).

The POD activity was measured based on the method of Chen et al. (2015) with some modifications. The reaction mixture consisted of 2.8 mL guaiacol (25 mM), 0.2 mL H_2O_2 (0.5 M), and 0.4 mL enzyme extract. The results were expressed as U kg^{-1} fresh weight, where one unit of POD activity was defined as an increase in absorbance by 0.01 per minute at 470 nm.

The CAT activity was determined as described previously (Zhang, Liu, He, & Zhu, 2015). The results were expressed as U kg^{-1} fresh weight, where one unit of CAT activity was defined as a decrease in absorbance by 0.01 per minute at 240 nm.

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The APX activity was analyzed according to Han et al. (2017) with slight modifications. The reaction mixture consisted of 2.5 mL sodium phosphate buffer (50 mM, pH 7.0), 0.1 mL enzyme extract, and 0.4 mL H_2O_2 (2 mM). The results were expressed as U kg^{-1} fresh weight, where one unit of APX activity was defined as a decrease in absorbance by 0.01 per minute at 290 nm.

2.6. Determination of MDA content and lipoxygenase (LOX) activity

The malondialdehyde (MDA) content was determined as described previously (Chen et al., 2015). The results were expressed as mmol kg^{-1} fresh weight. The LOX activity was assayed according to Surrey (1964). The results were expressed as U kg^{-1} fresh weight, where one unit of LOX activity was defined as an increase in absorbance by 0.01 per minute at 234 nm.

2.7. SEM and TEM observations

The blueberry cuticular wax was observed using scanning electron microscope (SEM) according to the method described previously (Chu, Gao, Cao, Fang, Chen, & Xiao, 2017). The ultrastructure of blueberry fruit was observed using transmission electron microscope (TEM) according to the method of Bu, Yu, Aisikaer, and Ying (2013).

2.8. Statistical analysis

The data were analyzed by one-way ANOVA with the IBM SPSS Statistics 21 software. The results are presented as mean ± SD (n = 3). The comparisons of means

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were evaluated using Fisher's LSD test at $P < 0.05$. The asterisks (*) show a significant difference between the control and wax-removed samples for each day.

3. Results and discussion

3.1. Effect of wax removal on the sensory and nutritional qualities and storability

3.1.1 Surface color

The acceptability of fruits' appearance is closely related to the surface color. For many fruit, such as blueberry, plum and grape berry, the L^* and b^* values could be affected by the natural wax layer (Saftner et al., 2008; Wisuthiphaet et al., 2014). As shown in Fig. 1B-D, after the wax removal from surface of the blueberry fruit，the L^* value was significantly decreased, whereas the b^* value was remarkably increased, indicating that the surface became much darker and less intense blue, giving the impression of poor fruit quality. This observation was in agreement with previous studies of wax bloom on European plum (Wisuthiphaet et al., 2014).

3.1.2 Weight loss, decay incidence, and firmness

Cuticular wax is assumed to be the first barrier against water loss and pathogenic attack (Bernard et al., 2013; Samuels et al., 2008). Significantly higher weight loss and decay incidence were observed in the blueberry fruit with the wax removed compared to those in the control fruit (Fig. 2A-B), implying that a natural wax layer on the fruit surface could prolong postharvest shelf-life to some extent.

Texture is also an important sensory quality, influencing marketability of various fruit. At the end of the storage period, firmness was markedly lower (by 21%) in the

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blueberry fruit with the wax removed than in the control fruit (Fig. 2C), which indicated that the existence of natural wax is helpful in maintaining the firmness of fruit and in delaying their softening.

3.1.3 TA, TSS, ascorbic acid, total phenolic, and total anthocyanin contents

The TA content in the control group decreased slowly throughout the cold storage period (Fig. 2D). However, a slight increase in the TA content was found for the fruit with the wax removed at later periods of storage, which can partially be attributed to the accumulation of organic acids produced by the spoilage microorganisms. Moreover, compared to the control, the removal of wax caused an obvious decline in the TSS, ascorbic acid, total phenolic, and total anthocyanin contents at later periods of storage (Fig. 2E-H), indicating that the existence of natural wax could contribute to maintenance of the flavor and nutrition of blueberry fruit during cold storage.

Cuticular wax influences the postharvest storage potential of fruits, such as water loss, decay susceptibility, and physiological disorders (Lara et al., 2014). In the present study, removal of natural wax accelerated the fruit senescence, which was reflected in the increase in postharvest water loss and decay incidence, as well as in the decline in sensory and nutritional qualities (Fig. 2).

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3.2. Possible mechanism for deterioration of postharvest quality in blueberry fruit by removing the wax

3.2.1 Effect of wax removal on the levels of $O_2^{\cdot-}$ and H_2O_2

A close relationship between the ROS content and fruit senescence has been reported earlier (Tian et al., 2013; Xia et al., 2016). ROS, such as $O_2^{\cdot-}$ and H_2O_2, are regarded as the by-products of normal cell metabolism (Sharma, Jha, Dubey, & Pessarakli, 2012). Under non-stress conditions, there is a stable equilibrium between the production and scavenging of ROS. However, under various stress conditions, this equilibrium is perturbed, leading to a considerable rise in the intracellular levels of ROS, which can induce irreversible damage to the cellular structures (Gill et al., 2010; Hajiboland, 2014). The fruit can encounter various types of physical or mechanical stress during the postharvest processing, such as during the fresh-cut process (Han et al., 2017; Li et al., 2017) and the decrowning process (Liu, He, Shen, Zhang, & Zhu, 2017).

In our study, compared to the control, the removal of blueberry wax induced a rapid increase in the $O_2^{\cdot-}$ and H_2O_2 content during storage (Fig. 3), especially at the early stages of storage, ultimately resulting in the over-accumulation of ROS. Similarly, a recent study reported that the postharvest detachment of pineapple crown increased the ROS accumulation and cell membrane damage (Liu et al., 2017).

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3.2.2 Effect of wax removal on the activities of antioxidant enzymes

To cope with the undesired accumulation of ROS, plants have developed an antioxidant defense system, which includes the antioxidant enzymes (e.g., SOD, POD, CAT, and APX) and nonenzymatic components (e.g., phenolic compounds, ascorbic acid, tocopherols, and carotenoids) (Hajiboland, 2014; Mittler, 2002). In this study, the increase in ROS content after the removal of wax triggered the activities of the antioxidant enzymes (Fig. 4) and the synthesis of phenolic compounds (Fig. 2G) in the early period of storage. Similar results were discovered in the studies on fresh-cut fruits and vegetables (Du, Avena-Bustillos, & Mchugh, 2012; Li et al., 2017). It is possible that low concentration of ROS, acting as second messengers, regulates the responses of plants under stress (Gill et al., 2010; Sharma et al., 2012).

Nevertheless, when the levels of ROS overwhelm the defense mechanisms, the plant cells are subjected to oxidative stress, which directly affects the normal cellular functioning (Mittler, 2002; Xia et al., 2016). With the increased duration of the storage of blueberry fruit, the activities of SOD, POD, CAT, and APX in the samples with the wax removed declined rapidly (Fig. 4), leading to weaker antioxidant defense capability compared to that in the control group. At the later storage period, the higher levels of $O_2^{\cdot-}$ in the samples with the wax removed might be attributed to the lower activity of SOD, which is responsible for converting $O_2^{\cdot-}$ into H_2O_2. Similarly, the higher level of H_2O_2 in the blueberry fruit, with the wax removed, during the later storage period was probably a result of lower activities of the antioxidant enzymes,

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including POD, CAT, and APX, all of which can remove the toxic H_2O_2 in plant cells (Gill et al., 2010; Mittler, 2002). Additionally, ascorbic acid and phenolic compounds, being the important nonenzymatic components of the antioxidative defense system, act as ROS scavenger (Hajiboland, 2014; Mittler, 2002). A decline in their contents in the blueberry fruit with the wax removed also implied weaker antioxidative ability (Fig. 2F-H). Taken together, at later period of storage, the rapid increases in ROS contents could be related to the decline in the activities of antioxidant enzymes and in the contents of nonenzymatic antioxidants contents. Weaker defense capability of the antioxidant system in the blueberry fruit with the wax removed lowered the scavenging of ROS, resulting in the accelerated membrane lipid peroxidation in fruit.

3.2.3 Effect of wax removal on the MDA content and the LOX activity

The high levels of ROS can lead to lipid peroxidation and can affect the normal cellular functioning (Jimenez et al., 2002; Sharma et al., 2012). The membrane lipid peroxidation is catalyzed by LOX, and can produce various small hydrocarbon fragments (Gill et al., 2010). MDA, being one of the final products of membrane lipid peroxidation, is usually regarded as an indicator of the severity of injury to cell membrane (Kotchoni, Kuhns, Ditzer, Kirch, & Bartels, 2006; Liu et al., 2017). In the present study, the MDA content and the LOX activity showed a gradual increase in both the groups of blueberry fruit (Fig. 5). Moreover, compared to the control, the higher levels of ROS in the blueberry fruit with the wax removed was in accordance with the enhanced LOX activity and MDA content, suggesting that the removal of

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